REMARKS

Applicants would like to thank Examiners Carlson and Snedden for their time and consideration in discussing the present application and pending claims during the Interview of May 15, 2003. The above-amendments and following remarks are submitted pursuant to the discussions during the Interview.

During the Interview the following issues were discussed and are addressed herein via the amendments and/or remarks.

1) Claim 11 -

a) During the Interview, the Examiners indicated that they would like claim 11, lines 1-2, to be amended for clarity to recite, "encoding an antimicrobial protein**, wherein said protein** ~~which~~ can be obtained from a fraction...." Claims 11, 30 and 31 have been amended as suggested by the Examiners.

b) The Examiners indicated that the first option of claim 11, wherein the protein encoded by the gene is defined by functional properties, is insufficient description of the encoded protein. The Examiners further indicated a concern that the first paragraph of claim 11 may encompass genes encoding "many" proteins. Claim 11 has been amended to delete the option of the first paragraph. As such, this issue is rendered moot regarding claim 11.

However, in as much as the issue may pertain to claim 30, the following remarks are submitted for consideration. Claim 30 is drawn to, an isolated gene encoding an antimicrobial protein, wherein the protein has the following properties:

i) the protein can be obtained from a fraction of an aqueous extract of *Lyophyllum shimeji* precipitated by the ammonium sulfate precipitation method,

ii) the protein has an antimicrobial activity at least against *Rhizoctonia solani* or *Pyricularia oryzae*, and

iii) the protein shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method.

Thus, while the gene of claim 30 is not necessarily obtained using *Lyophyllum shimeji* as the source of the gene, the encoded protein must be also present in *Lyophyllum shimeji*. The data of the specification demonstrates that there are only two suspected proteins in *Lyophyllum shimeji* that have the recited antimicrobial activity.

Figure 2 of the specification shows the relationship between the electrophoretic pattern of proteins from *Lyophyllum shimeji* and the associated antimicrobial activity. Only two bands of approximately 70 kDa and 65 kDa, respectively, correspond to the observed antimicrobial activity. See also page 36, line 21 to page

37, line 12 of the specification. As further noted in the specification it is believed that the 70 kDa band is a precusor protein of the 65 kDa protein. Thus, by recitation that the encoded protein can be isolated from *Lyophyllum shimeji*, the invention of claim 30 would not encompass a great number of encoded proteins.

c) The Examiners indicated that "homology" should be replaced with "identity" for the sake of clarity. The claims have been amended as suggested during the Interview to recite "identity." Applicants further note that the specification on page on page 13 details how to determine the percent homology or identity.

d) The Examiners indicated that the final paragraph of claim 11 is confusing with the position that this embodiment of the invention could be interpreted by one reading the claim as encompassing a gene encoding a protein having a fragment from the sequence of amino acid residues 76 to 618. Specifically, the Examiner indicated that "wherein said protein comprises a single polypeptide having a partial amino acid sequence of amino acid residues 76 to 618 of SEQ ID NO:2" could be interpreted as encompassing a protein that has only one or two amino acid residues that are the same as amino acid residues 76 to 618. Claim 11 has

been amended for clarity as indicated above, to recite that the encoded protein comprises at least amino acid residues 76 to 618 of SEQ ID NO:2.

2) Claim 19 -

During the Interview the Examiners indicated that 40 to 60% G+C is the normal content of the nucleotides and therefore felt recitation of "each domain has 40 to 60% of G+C" in claim 19 and 32 is superfluous. Claims 19 and 32 have been amended as suggested by the Examiners.

As the above-comments and amendments address and overcome the issues discussed during the Interview, allowance of the claims is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact MaryAnne Armstrong, PhD (Reg. No. 40,069) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

A marked-up copy of the amended portions of the specification and claims is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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GMM/MAA/
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MARKED-UP VERSION SHOWING CHANGES

IN THE CLAIMS

11. (Twice Amended) An isolated gene encoding an antimicrobial protein, ~~which can be obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method,~~

~~wherein said protein has an antimicrobial activity at least against Rhizoctonia solani or Pyricularia oryzae, and shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method; or~~

wherein said antimicrobial protein has an amino acid sequence of SEQ ID NO:2, or has 50% or more **identity** ~~homology~~ with said sequence and has an antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae; or

wherein said protein comprises ~~a single polypeptide having a partial amino acid sequence of~~ **an amino acid sequence of** amino acid residues 76 to 618 of SEQ ID NO:2, or a polypeptide having 50% or more **identity** ~~homology~~ with said ~~partial~~ amino acid sequence and having an antimicrobial activity against Rhizoctonia solani or Pyricularia oryzae, or a combination of these polypeptides.

13. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having a

50% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

14. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having a 60% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

15. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having a 70% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

16. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having an 80% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

17. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having a 90% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

18. (Twice Amended) The isolated gene according to Claim 11 encoding a protein having antimicrobial activity and having a 95% or more **identity** ~~homology~~ with the base sequence of SEQ ID NO:1.

19. (Twice Amended) An oligonucleotide for obtaining a gene encoding an antimicrobial protein originated from Lyophyllum shimeji produced by a process comprising:

selecting two domains from the base sequence of the gene of SEQ ID NO:1 wherein ~~said domains satisfy the following requirements: 1)~~ each domain consists of 15 to 30 bases~~; and 2) each domain has 40 to 60% of G+C;~~

preparing single-stranded DNAs having base sequences which are identical to the base sequences of said domains or complementary thereto, or preparing a single-stranded DNA mixture having degeneracy in the genetic code which ensures that the amino acid residues coded by said single-stranded DNAs are not changed; and optionally modifying the single-stranded DNAs while avoiding damage to the binding specificity to the base sequence of said gene encoding the antimicrobial protein.

30. (Amended) An isolated gene encoding an antimicrobial protein**, wherein said protein** ~~which~~ can be obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method, **and** wherein said protein has an antimicrobial activity at least against Rhizoctonia solani or Pyricularia oryzae, and shows the presence of components of about

70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method.

31. (Amended) An isolated gene encoding an antimicrobial protein**, wherein said protein** ~~which~~ can be obtained from a fraction of an aqueous extract of Lyophyllum shimeji precipitated by the ammonium sulfate precipitation method, **and** wherein said protein has an antimicrobial activity at least against Rhizoctonia solani or Pyricularia oryzae, and shows the presence of components of about 70 kDa and/or about 65 kDa in molecular weight in the SDS-PAGE method; and wherein said gene ~~according~~ has a base sequence of SEQ ID NO:1 or a base sequence which is complementary to a base sequence which hybridizes to SEQ ID NO:1 under stringent conditions of 6 x SSC, 45°C to 68°C (without formamide) or 25°C to 50°C (with 50% formamide).

32. (Amended) An oligonucleotide for obtaining a gene encoding an antimicrobial protein originated from Lyophyllum shimeji produced by a process comprising:

selecting two domains from the base sequence of the gene of SEQ ID NO:1, wherein ~~said domains satisfy the following requirements: 1)~~ each domain consists of 15 to 30 bases; ~~and 2) each domain has 40 to 60% of G+C~~; and

preparing single-stranded DNAs having base sequences which are identical to the base sequences of said domains or complementary thereto, or preparing a single-stranded DNA mixture having degeneracy in the genetic code which ensures that the amino acid residues coded by said single-stranded DNAs are not changed.